Antinociceptive effect of ambroxol in rats with neuropathic spinal cord injury pain

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ABSTRACT

Symptoms of neuropathic spinal cord injury (SCI) pain include evoked cutaneous hypersensitivity and spontaneous pain, which can be present below the level of the injury. Adverse side-effects obtained with currently available analgesics complicate effective pain management in SCI patients. Voltage-gated Na$^+$ channels expressed in primary afferent nociceptors have been identified to mediate persistent hyperexcitability in dorsal root ganglia (DRG) neurons, which in part underlies the symptoms of nerve injury-induced pain. Ambroxol has previously demonstrated antinociceptive effects in rat chronic pain models and has also shown to potently block Na$^+$ channel current in DRG neurons. Ambroxol was tested in rats that underwent a mid-thoracic spinal cord compression injury. Injured rats demonstrated robust hind paw (below-level) heat and mechanical hypersensitivity. Orally administered ambroxol significantly attenuated below-level hypersensitivity at doses that did not affect performance on the rotarod test. Intrathecal injection of ambroxol did not ameliorate below-level hypersensitivity. The current data suggest that ambroxol could be effective for clinical neuropathic SCI pain. Furthermore, the data suggest that peripherally expressed Na$^+$ channels could lend themselves as targets for the development of pharmacotherapies for SCI pain.

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1. Introduction

In patients with spinal cord injury (SCI), moderate to severe persistent pain is common and may worsen over time (Cruz-Almeida et al., 2005; Jensen et al., 2005; Siddall et al., 2003). Pain following SCI inhibits full participation in physical rehabilitation and greatly diminishes quality of life (Cruz-Almeida et al., 2005; Widerstrom-Noga et al., 2001). Although some pharmacotherapies provide pain relief to SCI patients, their regular usage is limited by both CNS and autonomic-mediated adverse side-effects (Turner et al., 2001; Warms et al., 2002). Thus, new analgesic drugs with the special needs of SCI patients in mind are needed.

Systemically administered voltage-gated Na$^+$ channel blockers, such as the local anesthetic lidocaine, are effective in ameliorating central as well as peripheral neuropathic pain. However, debilitating side-effects such as dizziness, somnolence and motor dysfunction coincide with the efficacy in this class of drug (Attal et al., 2004; Finnerup et al., 2005). Sodium channel subtypes expressed predominantly in primary afferent nociceptive neurons, but not cardiovascular tissue or the CNS, are promising targets for analgesic drug development with reduced potential for target-mediated adverse side-effects (Cummins et al., 2007; Krafte and Bannon, 2008; McGowan et al., 2009). The efficacy of peripherally acting drugs in general has not been evaluated in neuropathic SCI pain.

Ambroxol (2-Amino-3,5-dibromo-N-(trans-4-hydroxycyclohexyl) benzylamine) has been marked in the EU and other countries (but not in the U.S.) since 1978 as a mucolytic drug (Weiser, 2008). Ambroxol clears mucus resulting from respiratory disease by increasing the clearance of mucus from the respiratory tract (Fig. 1). One way by which ambroxol does this is decreasing the viscosity of mucus by increasing the release of surfactant from alveoli (Malerba and Ragnoli, 2008). An early clinical observation in patients with "airway disorders" reported improvement in "painful complaint" with ambroxol applied via an inhaler to the oropharynx (Hoffmann, 1978). More recent double-blinded, placebo-controlled clinical trials in patients with acute pharyngitis confirmed pain relief with ambroxol treatment (de Mey et al., 2008; Fischer et al., 2002). Only recently, however, has a mechanism been proposed to explain the local anesthetic effect, which is unrelated to the mucolytic effect. Ambroxol has been shown to decrease the tetrodotoxin-resistant (TTX-R) Na$^+$ channel current found in dorsal root ganglia (DRG) neurons and, furthermore, demonstrated antinociceptive effects in rat models of peripheral nerve injury, inflammation and in the formalin test (Gaida et al., 2005; Leffler et al., 2010; Weiser, 2006, 2008). In addition, since ambroxol does not cross the blood–brain barrier, the antinociceptive effect could be mainly due to blocking of Na$^+$ channels expressed in primary afferent nociceptors.

Some of the symptoms of chronic SCI pain resemble that of peripheral neuropathic pain, with the presence of spontaneous pain and hypersensitivity to cutaneous stimulation (Felix et al., 2007; Yezierski, 2000). Curiously, neuropathic symptoms may exist not only at-level but also below the level of SCI. A number of genetic and

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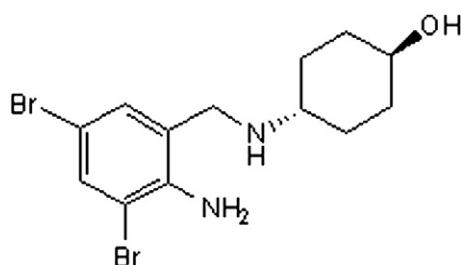*E-mail address:* ahama@med.miami.edu (A.T. Hama).

Fig. 1. Chemical structure of ambroxol.

neurochemical changes in the spinal cord following SCI have been implicated in the initiation and maintenance of the chronic SCI pain state, resulting in increased spontaneous and evoked activity in dorsal horn neurons below the level of injury. Changes in glial function following SCI, observed several segments caudal to the lesion, have also been implicated in the maintenance of neuropathic SCI pain (Hains et al., 2002, 2003; Hulsebosch et al., 2009; Yezierski, 2000; Zhang et al., 1994). The development of treatments for neuropathic SCI pain has focused on these central changes.

Changes in peripheral nociceptor Na^+ channel function and distribution have been reported in rat chronic pain models and in humans following nerve injury (Coward et al., 2000; Cummins et al., 2007; Hudmon et al., 2008; Yiangou et al., 2000). Compounds acting at peripheral sites may be clinically more favorable in terms of decreased side-effects (Krafte and Bannon, 2008). Changes in peripheral nerve function, many segments distal to a spinal lesion, have also been reported (Carlton et al., 2009; Lin et al., 2007). As with direct injury to the nerve, it is possible that gene and protein expression is also changed — Na^+ channel distribution within apparently undamaged peripheral nerves could also be altered following SCI.

The positive pre-clinical findings from various models of peripheral tissue-injury-mediated pain suggests that ambroxol could be a clinically useful analgesic. Pharmacokinetic data in both humans and animals have been generated and ambroxol has an extensive clinical safety record (Table 1). However, since few drugs that are efficacious in these models are efficacious in a rat model of neuropathic SCI pain, it is not known if efficacy in peripheral tissue-injury models will translate to efficacy in clinical neuropathic SCI pain (Hama and Sagen, 2007). Thus, the current study evaluated the efficacy of ambroxol in rats with neuropathic SCI pain.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Harlan, IN) were used and had free access to food and water. Rats were housed in pairs, before and after surgery, and were kept in the vivarium on a 12 hr light:dark cycle.

Table 1
Pharmacokinetic properties of orally administered ambroxol.
Pharmacological data were compiled from Gaida et al. (2005), Kubo et al. (2004), Malerba and Ragnoli (2008), and Miyazaki et al. (2005).

	Rat	Human
Peak plasma concentration (C_{max}), μM		
300 mg/kg	2.2	–
1000 mg/kg	7.2	–
30 mg	–	0.2
Time to peak C_{max} (T_{max}), hours	0.5	1.6
Half-life ($T_{1/2}$), hours	n.a.	9.7
Mean residence time, hours	2	9.2
Absolute bioavailability ($F\%$)	n.a.	79
50% lethal dose (LD_{50}), mg/kg	13,400	–

C_{max} was measured 90–130 min post-dosing Gaida et al. (2005).
n.a., not available.

Rats were between 100–150 g upon arrival to the vivarium. The procedures were reviewed and approved by the University of Miami Animal Care and Use Committee.

2.2. Spinal cord injury surgery

A previously described procedure utilizing a micro-vascular clip was used to induce a mid-thoracic SCI (Hama and Sagen, 2007). Rats were anesthetized with isoflurane in O_2 and the back was shaved from the cervical to lumbar vertebral level. Rats were maintained on isoflurane in O_2 and placed prone on the surgery table. Using aseptic technique, the paraspinal muscles were bluntly dissected to expose vertebrae T6–T7. Mid thoracic spinal segment, approximately T6–T7, was exposed via laminectomy. A micro-vascular clip (15–20 g; Harvard Apparatus, MA) was vertically applied over the exposed spinal segment and compressed the cord for 1 min. The clip was then removed and the muscles and skin closed. Rats were returned to their home cages to recover. No antibiotic or post-operative analgesic was administered to these rats. Rats with a SCI were tested beginning 4 wks following spinal surgery. At the end of the drug study rats were humanly euthanized with CO_2 overdose.

2.2.1. Intrathecal catheters

Using aseptic technique, indwelling intrathecal (i.t.) catheters were implanted in 6 rats three weeks following SCI surgery (Yaksh and Rudy, 1976). Rats were anesthetized with isoflurane/O_2 and the atlanto-occipital membrane was exposed. A catheter was introduced into the subarachnoid space and the internalized portion ended at the lumbar enlargement (ReCathCo, PA). Rats were tested one week after intrathecal surgery (i.e. four weeks after SCI surgery). The SCI rats had impaired hind limb function prior to i.t. surgery (see Results section), but not complete paralysis. Thus, rats that displayed complete hind limb paralysis following i.t. surgery were excluded from the study. Rats were tested on two different days separated by at least 24 h. On day 1, rats were tested with heat and on day 2 rats were tested with von Frey filaments (see below).

At the end of behavioral testing, 5 μl of 1.5% (i.e. 75 μg) lidocaine HCl (in saline) was i.t. injected to confirm the placement of the internal end at the level of the lumbar enlargement. A bi-lateral flaccid paralysis of the hind limbs confirmed proper catheter placement.

2.3. Behavioral testing

Because of the need to acclimate rats to different chambers prior to testing, it was not possible to test each drug-treated rat with both mechanical and heat stimuli. Thus, separate groups of rats were assigned to a particular stimulus.

2.3.1. Mechanical hypersensitivity

Rats were placed in clear Plexiglas containers resting on an elevated wire mesh floor and allowed to acclimate to the chamber for at least 30 min. Eight specific calibrated von Frey filaments were used via the up-down method to determine the withdrawal threshold (grams) of the hind paws (Stoelting Co., IL). The filaments were applied to the plantar hind paw, bent slightly and held on the rat skin for 6 s (Chaplan et al., 1994). The 50% withdrawal threshold was calculated from the pattern of responses to the filaments (Chaplan et al., 1994). An upper limit of 15 g was used.

The amount of force needed to evoke a withdrawal was reported in "grams" in the current study, although force is usually reported in "Newtons." There is a lack of consistency in the use of units of force needed to evoke a response (withdrawal) across clinical and pre-clinical studies (Attal et al., 2004; Gaida et al., 2005; Jarvis et al., 2007; Wallace et al., 2000). In keeping with the original description of the method (Chaplan et al., 1994) as well as to facilitate comparison across the majority of pre-clinical pharmacological studies, the current study will report thresholds in grams.

2.3.2. Heat hypersensitivity

A commercially available apparatus was used to assess sensitivity of the plantar hind paws to a brief noxious heat stimulus (Hargreaves et al., 1988). A rat was placed in a Plexiglas enclosure that rested on an elevated glass floor. After an acclimating period of 30 min, an infrared emitter that was under the glass floor was positioned directly beneath the mid-plantar hind paw. A hind paw withdrawal stopped the infrared stimulus. The length of time between the onset of the stimulus and the paw withdrawal is the withdrawal latency (seconds). The average of three trials per paw was used to report the final withdrawal latency. Testing of the same paw was separated by about 5 min. To prevent tissue damage, a cut-off of 20 s. was used.

Following measurement of baseline withdrawal threshold or latency, rats were dosed p.o. with either drug or vehicle and tested 30, 60, 90, 120, 150 and 180 min post-dosing.

For rats with i.t. catheters, rats were tested 30, 60, 90, and 120 min post-injection.

2.3.3. Rotarod test

The effect of ambroxol on coordinated motor function in uninjured rats was assessed with an accelerating rotarod apparatus (Columbus Instruments, OH; (Hama et al., 2003)). A group of uninjured rats were trained once a day for two days on the apparatus prior to testing. The rotarod accelerated 5–30 rpm over 60 s. A baseline latency to fall (seconds) was recorded prior to p.o. dosing with either 1000 mg/kg ambroxol or vehicle. Rats were tested 60 min following dosing.

2.3.4. The Basso, Beattie, Bresnahan (BBB) Locomotor Rating Scale

To assess possible acute effects of ambroxol on spontaneous hind limb function in SCI rats, two blinded observers scored SCI rats treated with either 1000 mg/kg ambroxol or vehicle 60 min post-dosing, using the Basso, Beattie, Bresnahan (BBB) Locomotor Rating Scale, a standardized rating system that has been developed to assess hind limb function following a SCI (Basso et al., 1995). Rats were placed in the center of a 1.2 m diameter arena, allowed to continuously locomote and were rated within a four minute test period. In this assessment, multiple factors were included, such as hind paw/fore paw coordination, toe clearance from the surface and paw position. These factors were considered when a assigning a BBB rating score.

The standardized scale ranges from 0 to 21. In normal, uninjured rats, the BBB score is 21, which is characterized by "consistent plantar surface paw stepping and coordinated gait" and "consistent toe clearance," among other observations. By contrast, a score of 0 indicates complete absence of hind limb function. Increasing levels of hind limb function, such as (and not limited to) movement at the ankle, knee or hip joints, toe clearance and supporting of the weight with the hind limbs increases the BBB score.

2.4. Drugs

Ambroxol HCl (Sigma-Aldrich Co., MO) was dissolved in a vehicle of 10% Tween-80 in water. Ambroxol and its vehicle were administered p.o. in a volume of 5 ml/kg. Ambroxol up to a concentration of 60 mg/ml was a clear solution. However, over this concentration, ambroxol was administered as a suspension. Doses were based on a previous pharmacokinetic study, partially summarized in Table 1, and a previous behavioral study (Gaida et al., 2005).

In i.t. studies, the dose of ambroxol was 300 µg in a volume of 5 µl. Following injection of either ambroxol or vehicle, an additional 5 µl of vehicle was injected to flush the catheter.

2.5. Statistical analysis

Data are expressed as mean ± S.E.M. The effect of drug treatment over time was evaluated using a repeated measure two-way ANOVA (SigmaStat 3.1), followed by a Student–Newman–Keuls test for post-hoc comparisons. To compare the effect of ambroxol against vehicle administration in the rotarod test and BBB rating, the data were analyzed using a one-way ANOVA with Dunnett's test for post-hoc comparison. Statistical significance was taken at $P < 0.05$.

At the time point at which maximal efficacy was observed (i.e. 60 min post-dosing for mechanical hypersensitivity and 90 min post-dosing for heat hypersensitivity), behavioral data were normalized in terms of baseline. Mechanical withdrawal thresholds were converted to a percent maximum possible effect (MPE):

$$\% \text{ MPE} = (\text{Drug threshold} - \text{Baseline threshold})$$
$$\div (15 \text{ g} - \text{Baseline threshold}) \times 100$$

Heat withdrawal latencies were converted to a percent reversal:

$$\% \text{ Reversal} = (\text{Drug latency} - \text{Baseline latency})$$
$$\div (\text{Pre} - \text{injury latency} - \text{Baseline latency}) \times 100$$

3. Results

3.1. Antinociceptive effect of ambroxol

The mechanical withdrawal threshold in uninjured rats was 15 g. After SCI, prior to p.o. dosing, the mean ($\pm$ S.E.M.) hind paw withdrawal threshold was 2.0 ± 0.2 g, indicating significant below-level mechanical hypersensitivity. The highest dose of ambroxol, 1000 mg/kg, significantly reversed mechanical hypersensitivity beginning 30 min after dosing and persisted for at least 3 h post-dosing ($P < 0.05$ vs. vehicle; Fig. 2A). Other doses of ambroxol did not significantly increase thresholds. By contrast, vehicle did not significantly alter withdrawal threshold at any time point. The maximum possible effect of ambroxol at 60 min post-dosing was 72% (Fig. 2B). Rats were tested 24 h following dosing and withdrawal thresholds were as they were on the previous day prior to dosing.

The heat withdrawal latency in uninjured rats was 11.9 ± 0.2 s. Following SCI, but prior to dosing, the withdrawal latency was 7.8 ± 0.2 s, indicating significant heat hypersensitivity ($P < 0.05$ vs. uninjured; Fig. 3A). Beginning about 60 min post-dosing, increases in withdrawal latencies was observed in rats treated with 300 mg/kg ambroxol. A partial reversal of heat hypersensitivity was observed at 90 min with doses of 100 mg/kg (65%) and 300 mg/kg (61%) ($P < 0.05$ vs. vehicle; Fig. 3B). The antinociceptive effect at these doses persisted up to 120 min post-dosing. By contrast, vehicle treatment did not significantly increase latencies at any time point. Rats were tested 24 h following dosing and withdrawal latencies were as they were on the previous day prior to dosing.

There was no significant effect at any time point following i.t. injection of ambroxol or vehicle on either heat or mechanical hypersensitivity in SCI injured rats. Fig. 4 shows the lack of effect of ambroxol and vehicle treatment at 30 min post-i.t. injection ($P > 0.05$ vs. vehicle).

As a general observation, i.t. injection of 75 µg lidocaine led to a rapid bi-lateral hind limb flaccid paralysis. However, i.t. injection of ambroxol did not.

3.2. Rotarod test

Prior to treatment, uninjured rats maintained positions on the rotarod for the entire 60 sec period. One hour after dosing, neither ambroxol nor vehicle significantly decreased time spent on the rotarod ($P > 0.05$; Table 2).
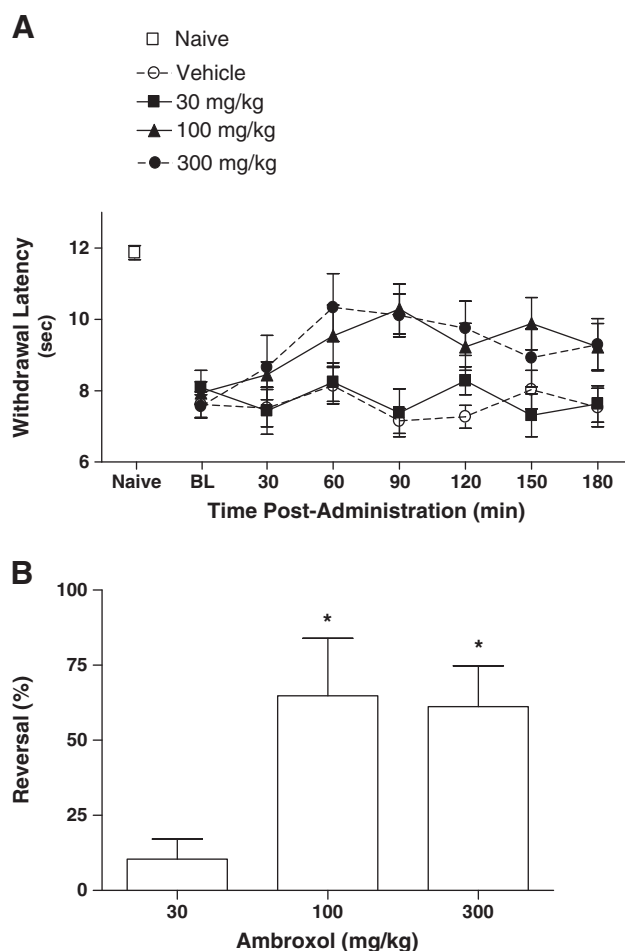
A

B
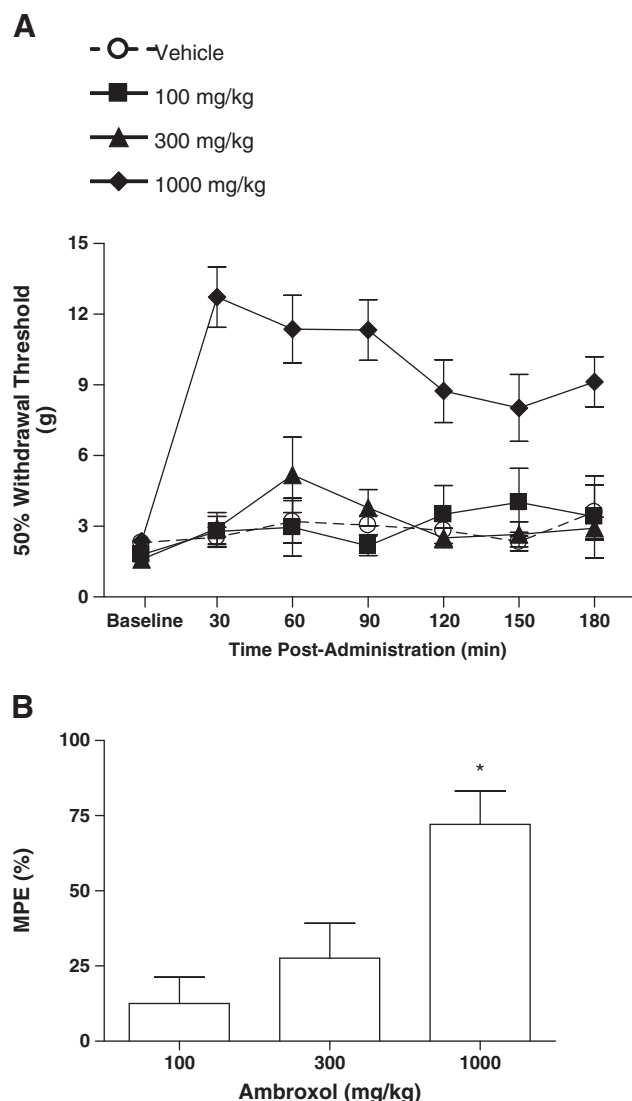
Fig. 2. Effect of ambroxol on below-level mechanical hypersensitivity in rats with a spinal cord injury. The rats were tested prior to ("baseline") and following p.o. dosing of either ambroxol or vehicle. (A) The effect over time of ambroxol on withdrawal threshold (g). The highest dose (1000 mg/kg) significantly increased withdrawal thresholds at all tested time points (*$P<0.05$ vs. vehicle and baseline). (B) Percent maximum possible effect (MPE) of ambroxol 1 h post-dosing (*$P<0.05$ vs. vehicle). Data are expressed as mean ± S.E.M. N = 7/group.

3.3. BBB rating

The pre-SCI BBB rating of uninjured rats was 21. Four weeks after SCI and prior to dosing, the BBB rating of the SCI rats was $9±1$, indicating occasional weight-supported, dorsal hind paw stepping and weight-supported only while stationary. One hour following dosing, neither ambroxol nor vehicle significantly altered BBB scores ($P>0.05$; Table 2). Thus, an acute dose of ambroxol does not improve hind limb impairment due to SCI.

4. Discussion

The current study uncovered an antinociceptive effect of the mucolytic and local anesthetic drug ambroxol in a rat model of neuropathic SCI pain. Significant antinociception was obtained following oral dosing, reversing below-injury level tactile and noxious heat hypersensitivity. The highest tested dose of ambroxol did not lead to impairment of coordinated locomotion as assessed by the rotarod test. It has been previously reported that in vitro application

A

B

Fig. 3. Effect of ambroxol on below-level heat hypersensitivity in rats with a spinal cord injury. The rats were tested prior to ("BL," baseline) and following p.o. dosing of either ambroxol or vehicle. Note the decreased withdrawal latency of spinal cord-injured rats compared to "Naïve" rats. (A) Effect of ambroxol over time on withdrawal latency (seconds). (B) Percent reversal of hypersensitivity of ambroxol at 90 min post-dosing (*$P<0.05$ vs. vehicle). The data are expressed as mean ± S.E.M. N = 6/group.

of ambroxol potently blocked voltage-gated Na^+ channel currents in DRG neurons, suggesting the antinociceptive effects in vivo is due to blocking of primary afferent nociceptor function. The lack of effect following i.t. injection of ambroxol excludes a direct lumbar spinal site of action in the systemic effect of ambroxol. The findings indicate that, not only could ambroxol be useful in treating neuropathic SCI pain, but also suggest that Na^+ channels expressed on primary afferents could be useful drug targets for neuropathic SCI pain.

The mechanical and ischemic damages subsequent to SCI evoke changes in gene expression and neurochemistry in both neurons and glia, such as increased transcription factor expression, elevated concentrations of excitotoxic neurotransmitters, growth factors and cytokines (Hulsebosch et al., 2009). In addition to local changes in spinal dorsal horn, alterations in tissue homeostasis may spread distally from the epicenter to adjacent spinal cord segments and to brain somatosensory nuclei (Cummins et al., 2007; Yezierski, 2000; Zhou et al., 2002). Interestingly, nerve function outside the CNS may also be altered following an acute SCI, which could also underlie SCI-induced neuropathic pain (Carlton et al., 2009; Lin et al., 2007).

A possible point of mechanistic convergence of neuropathic pain due to injury of the peripheral or central nervous system could be the injury-induced abnormal and persistent function of dorsal horn wide-dynamic range (WDR) neurons, which respond to both noxious and innocuous stimuli (Carlton et al., 2009; Millan, 1999). Following tissue injury, these neurons display greatly exaggerated and long-lasting
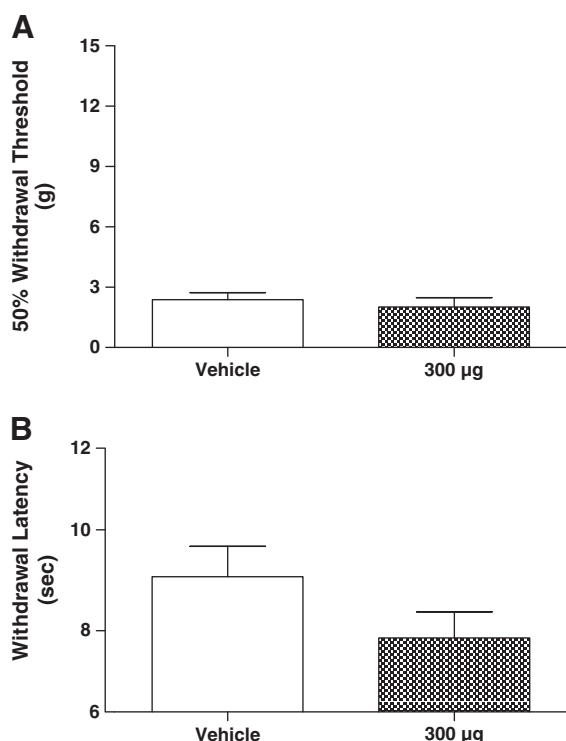
Fig. 4. Effect of intrathecal ambroxol on below-level hypersensitivity in rats with a spinal cord injury. Lumbar intrathecal (i.t.) injection of 300 μg of ambroxol had no significant effect on either mechanical (A) or heat (B) hypersensitivity. There was no effect of i.t. injection of vehicle. The data are taken from rats 30 min following i.t. injection. There was a lack of effect at other time points as well. The data are expressed as mean ± S.E.M. N = 3/group.

responses to noxious and innocuous cutaneous stimuli and increased spontaneous activity, which have been suggested to underlie hypersensitivity and spontaneous pain. Sodium channels expressed in peripheral nerves appear to be crucial in the maintenance of persistent injury-induced pain. Injection of a non-selective or a subtype selective Na$^+$ channel blocker into lumbar DRG in rats with a peripheral injury reduced evoked WDR activity and cutaneous hypersensitivity (McGaraughty et al., 2008; Omana-Zapata et al., 1997; Sotgiu and Biella, 2000). These findings suggest that peripheral, pre-synaptic block of Na$^+$ channels is sufficient in attenuating both abnormal WDR activity and nociception. It is possible that a similar treatment could ameliorate SCI-induced hypersensitivity.

Clinical use of non-selective Na$^+$ channel blockers lidocaine and its analogue mexiletine appear to be effective on some neuropathic pain symptoms, the efficacy being non-dose dependent and the centrally-mediated side-effects are not well tolerated (Attal et al., 2004; Ferrante et al., 1996; Finnerup et al., 2005; Wallace et al., 2000). In a

Table 2
Effect of ambroxol on motor function in the rat.

	Pre-dose	Post-dose
Rotarod test (seconds)		
Vehicle	60 (0.0)	60 (0.0)
Ambroxol	60 (0.0)	60 (0.0)
BBB		
Vehicle	9.9 (0.7)	9.4 (1.2)
Ambroxol	7.5 (1.2)	8.3 (0.7)

The rats were tested 1 h following oral dosing. The latency to fall (seconds) in uninjured rats in the rotarod test was measured. BBB locomotor scores were measured on a scale of 0–21 in SCI rats. Neither vehicle nor ambroxol affected performance in either test. The data are mean (S.E.M.), N = 4/group.

previous study, the highest tested dose of mexiletine (75 mg/kg) did not ameliorate either mechanical or heat hypersensitivity in SCI rats (Hama and Sagen, 2007). Furthermore, a slight increase in the dose of mexiletine (100 mg/kg) induced lethal convulsions in about 50% of the rats. Because of the central and peripheral adverse effects of non-selective Na$^+$ channel block, systemically administered local anesthetic drugs may not be ideal for pain relief. Compared to currently available Na$^+$ channel blockers, ambroxol is very well tolerated in humans and animals (Weiser, 2008). No effect was observed in the current study on coordinated locomotion following the highest tested dose of ambroxol, which was well below the LD$_{50}$ (Table 1; Püschmann and Engelhorn, 1978).

Interestingly, initial pre-clinical characterization of ambroxol did not uncover a robust antinociceptive effect (Püschmann and Engelhorn, 1978). Ambroxol was tested in mice using the tail-pinch and acetic acid-induced writhing assays. The dose range tested was 50–200 mg/kg (p.o.). It is possible that the mice were under-dosed. A higher dose was later used (1000 mg/kg) with no significant antinociceptive effects in the tailflick and hot plate assays (Gaida et al., 2005). This same dose, however, demonstrated efficacy in tissue-injury models of pain. Thus, dosing was not likely an issue.

The lack of a positive antinociceptive effect in uninjured rats suggests that these assays (e.g. tailflick, hot plate) are weakly able to detect antinociceptive effects of systemic local anesthetics (Wiesen-feld-Hallin and Lindblom, 1985). Other local anesthetic drugs have demonstrated variable effects in suppressing the electrically induced mono-synaptic (c-fiber mediated) or poly-synaptic (myelinated afferents mediated) reflexes in vivo (Woolf and Wiesenfeld-Hallin, 1985). Ambroxol did not alter either of these. The only in vivo assay that indicated that ambroxol had local anesthetic activity was in the suppression of the rabbit corneal reflex, which is c-fiber mediated (Püschmann and Engelhorn, 1978). Clinical studies confirmed a robust anesthetic effect based on the suppression of painful cough, also c-fiber mediated, an unexpected finding since ambroxol was developed and tested for mucolysis, a non-neurological indication (de Mey et al., 2008; Fischer et al., 2002; Hoffmann, 1978). The experience with ambroxol, a mucolytic and local anesthetic drug, demonstrates the need for careful selection of in vivo assays for Na$^+$ channel blockers in particular and analgesic drugs in general for discovery. The experience also suggests the need to be prepared for the serendipitous ("chance favors the prepared mind").

It was previously shown that ambroxol potently blocks the TTX-R current in vitro, which is mediated by Na$_v$1.8 and Na$_v$1.9, typically found in primary afferent nociceptors (Weiser, 2006). A more recent study confirmed ambroxol preferentially blocking Na$_v$1.8 channels compared to heterologously expressed neuronal channel subtypes Na$_v$1.3 and Na$_v$1.7 (Leffler et al., 2010). Unlike local anesthetic drugs, however, ambroxol does not cross the blood–brain barrier. To ascertain a possible spinal site of action, ambroxol was injected into the lumbar intrathecal space — there was no effect on below-level hypersensitivity. Thus, one possible mechanism of ambroxol's effect in the current and previous in vivo studies is through block of peripherally expressed Na$^+$ channels, particularly those channels expressed following injury.

In the current study, ambroxol ameliorated below-level mechanical and noxious heat hypersensitivity in SCI rats. Ambroxol's effect on mechanical hypersensitivity was restricted to the highest tested dose, similar to that observed in peripheral nerve-injured rats (Gaida et al., 2005). The all-or-none effect with Na$^+$ channel blocking drugs has also been clinically observed (Ferrante et al., 1996). The high dose-only effect could be related to the limited distribution of Na$_v$1.8 in large-diameter mechano-sensitive primary afferents in contrast to the abundant expression of Na$_v$1.8 in small-diameter nociceptors. Anti-nociceptive effects are observed with Na$^+$ channel subtype selective compounds, but not ambroxol, in uninjured rats with tests of acute nociception (Jarvis et al., 2007; Weiser, 2008). An increase in Na$_v$1.8 in

peripheral nociceptor axons following hind paw inflammation appears to underlie the increased potency of the $Na_v1.8$ selective compound, compared to that of uninflamed rats (Coggeshall et al., 2004). An increased expression $Na_v1.8$ and other Na^+ channels in SCI rats could explain the differential doses needed and efficacy between noxious heat and tactile hypersensitivity.

The current in vivo data demonstrated a long-lasting antinociception following p.o. dosing. Maximal plasma concentration of ambroxol is rapidly attained following oral dosing and the mean residence time is 2 h (Table 1). However, efficacy was observed for at least 2–3 h. A similar prolonged analgesia has been reported following lidocaine administration (Chaplan et al., 1995). Furthermore, the plasma concentration of lidocaine (2.6 μM) needed for a prolonged analgesia was much lower than that needed to block DRG neuron Na^+ channels in vitro, usually in the mM range, and much lower than that needed to block nerve conduction (Weiser, 2006). The mechanism explaining the prolonged analgesia of local anesthetics has yet to be fully elaborated. Perhaps the effect in the current study was due to a novel blocking effect on Na^+ channels or an active metabolite. Ambroxol has been shown to block other ion channels at high concentrations and has anti-inflammatory properties, which could also underlie the antinociceptive effect in the current study. However, the importance of these mechanisms in the antinociceptive of ambroxol has yet to be established (Malerba and Ragnoli, 2008).

Because of the added health burdens due to SCI, a peripherally acting analgesic would be ideal in these patients (Turner et al., 2001; Warms et al., 2002). A number of subtype selective compounds that block primary afferent-expressed Na^+ channels have been developed with the goal of pain relief without the liability obtained with non-selective, brain penetrant Na^+ channel blockers (Krafte and Bannon, 2008; McGowan et al., 2009). As a part of the drug discovery process, the safety of these novel chemical entities will need extensive and time-consuming evaluation. In the mean time, drugs that are already marketed, such as ambroxol, could be considered for clinical use (Chong and Sullivan, 2007).

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